THE BULLETIN OF Mathematical BIOPHYSICS

SEPTEMBER 1943

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THE UNIVERSITY OF CHICAGO PRESS · CHICAGO · ILLINOIS
VOLUME 5 · · · · · NUMBER 3

THE BULLETIN OF MATHEMATICAL BIOPHYSICS

EDITED BY N. RASHEVSKY

The Bulletin is devoted to publications of research in Mathematical Biophysics, as described on the inside back cover.

THE BULLETIN is published by the University of Chicago at the University of Chicago Press, 5750 Ellis Avenue, Chicago, Illinois, quarterly, in March, June, September, December. ¶The subscription price is $2.50 per year, the price of single copies is 75 cents. Orders for service of less than a full year will be charged at the single-copy rate. ¶Patrons are requested to make all remittances payable to The University of Chicago Press in postal or express money orders or bank drafts.

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BUSINESS CORRESPONDENCE should be addressed to The University of Chicago Press, Chicago, Ill.

COMMUNICATIONS FOR THE EDITOR and manuscripts should be addressed to N. Rashevsky, Editorial Office of the Bulletin of Mathematical Biophysics, 5822 Drexel Avenue, Chicago, Ill.

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PRINTED BY THE DENTAN PRINTING COMPANY · · · COLORADO SPRINGS, COLORADO

BULLETIN OF
MATHEMATICAL BIOPHYSICS
VOLUME 5, 1943

THE KINETICS OF ENZYME INACTIVATION

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The course of hydrolysis of β-glycerophosphate catalyzed by a group of different enzyme extracts, both with and without the addition of Mg, with and without preincubation of the enzyme, has been studied and the results discussed on the basis of a mathematical analysis. In all the extracts, it appears that two distinct and independently acting constituent enzymes—or perhaps "principles" of the same enzyme—are present, one acting much more rapidly but also more rapidly inactivated than the other. Storage in the refrigerator changes markedly the behavior of both constituents, though in different ways. There is evidence that in some cases an enzyme is limited in its hydrolytic "capacity" in the sense that after an enzyme molecule has decomposed a definite number of substrate molecules, it thereafter becomes entirely passive. Further, there is evidence, in the case of one extract, that the roles of catalytically more and less active constituents in the absence of Mg are reversed in its presence. Finally, a damped periodicity is found which indicates the presence of two factors of an unknown sort which influence and are influenced by the inactivation of the enzyme.

The heat inactivation of enzymes in the absence of substrate is a well known phenomenon. So also is the decline in the rate of enzyme reactions during the course of this reaction. Shutz's law takes account of this decline but is purely empirical and postulates no mechanism. Attempts to derive this law, or approximations to it have involved diverse assumptions, including the notion of an enzyme-inhibitor complex (Arrhenius, 1907) and slow diffusion of the substrate, (Moelwyn-Hughes, 1936).

In some sense, whether by mechanical blocking (Moelwyn-Hughes), or whatever, the enzyme seems to be undergoing an inactivation during the course of the reaction, and this process is distinguished—and presumably different—from the process of heat inactivation undergone in the absence of the substrate. It is reasonable to suppose that a comparison of the two processes for a given enzyme should reveal some properties of this enzyme not apparent from a consideration of one of these processes alone.

Hydrolysis of β-glycerophosphate by a group of phosphates has been studied for the purpose suggested. In general, the substrate concentration has been made sufficiently high to justify our neglecting this as a significant variable. Also, the concentration of the enzyme extract has been taken low enough so that the reaction rate is simply proportional to this concentration. In all cases the solution has been strictly buffered at a *pH* of 9.1, with a maximum variation of .02, and the temperature has been 37° with maximum variation of .2. The procedure has been to incubate the extract in the absence of the substrate, for a period of time, and to measure in gammas the amount of inorganic phosphates produced in a standard solution at varying periods after combining with the substrate. Throughout, we shall denote by $p_\tau(t)$ the amount of inorganic phosphates produced after t hours of the reaction catalyzed by an extract which had been preincubated for τ hours. A detailed report of the experiments will be published elsewhere.

A priori the extract might consist of any number of constituent enzymes, X, Y, $\cdots$, acting independently. Actually, to interpret the results obtained, the simplest supposition seemed to be that all the extracts studied contained exactly two such constituents, with markedly different behavior and in markedly different proportions. In only one case was the presence of a second constituent doubtful, though in no case did it reveal itself with any certainty until the reaction had run for a period of 24 hours or longer.

If there were one enzyme alone, on the proportionality assumption we could write

$$\frac{dp}{dt} = x$$

where x represents the product of the number of moles of enzyme present by the number of moles of substrate decomposed in unit time by one mole of X. Since we have no means of separating these two factors, we shall speak loosely of x as measuring the "amount" of X. If two enzyme constituents are acting independently, we have in place of the above equation,

$$\frac{dp}{dt} = x + y. \tag{1}$$

If X and Y are inactivated monomolecularly by heat at rates h and k, we have

$$dx/d\tau = -hx, \quad dy/d\tau = -ky, \tag{2}$$

whence

$$x_\tau = x_0\, e^{-h\tau}, \quad y_\tau = y_0\, e^{-k\tau}. \tag{3}$$

If the rates of inactivation during the reaction are α and β, the process being again monomolecular, then

$$dx/dt = -\alpha x, \quad dy/dt = -\beta y, \tag{4}$$

and, in view of equation (1),

$$p_\tau(t) = x_\tau(1 - e^{-\alpha t})/\alpha + y_\tau(1 - e^{-\beta t})/\beta \,. \qquad (5)$$

In case β is small, the last equation becomes

$$p_\tau(t) = x_\tau(1 - e^{-\alpha t})/\alpha + y_\tau t \,. \qquad (6)$$

These equations result from the simplest suppositions we can make, and we proceed to consider the results in the case of one extract for which we have the most abundant data. Some of the observations were repeated after the enzyme had been stored in the refrigerator for a period of some months, and the behavior was seen to change somewhat during this time.

Data from the fresh enzyme, with $\tau = 0$ and $\tau = 24$, were found to follow equation (6) with

$$\alpha = 0.9 \text{ hour}^{-1}; \quad h = k = 0.029 \text{ hour}^{-1}; \quad x_0/\alpha = 160; \quad y_0 = 3.3 \,.$$

Here and throughout, α, h and k are measured in inverse hours, while p, x and y are in arbitrary units. The dimension of p is that of a mass, the dimension of x and y—mass per unit volume. Measurements of p were made in this as in all other cases, for $t = 1, 2, 4, 8, 12, 24, 48, 72$ and 96 hours. Since

$$0.029 = (\log_e 2)/24 \,,$$

these values of h and k amount, according to equation (3), to setting $x_{24} = x_0/2$, $y_{24} = y_0/2$, so that, by equation (6):

$$p_0(t) = 2\, p_{24}(t) \,.$$

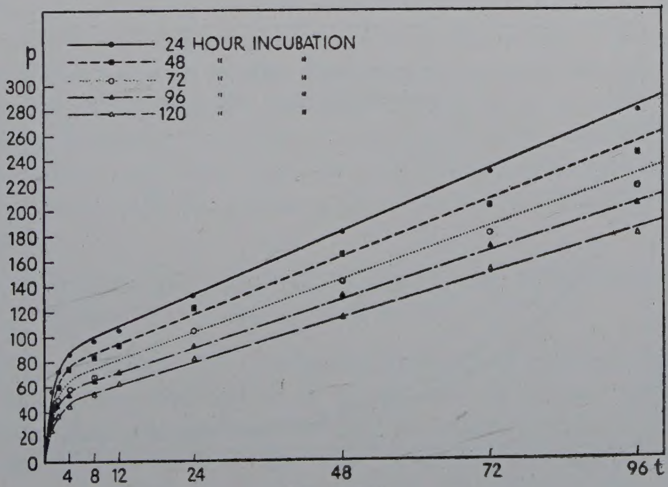

FIGURE 1

Thus the heat inactivation of the X and Y constituents proceeded at the same rate, whereas during the reaction, X broke down at about 30 times this rate, Y not to any measurable degree.

After being stored for a time in the refrigerator, these experiments were repeated and others performed, incubation being allowed to run also for 48, 72, 96 and 120 hours (Figure 1). All curves satisfied equation (6), and except for p_0, it was found that

$$\alpha = 0.8 \text{ hour}^{-1}; \quad h = 0.00723 \text{ hour}^{-1}; \quad k = .00349 \text{ hour}^{-1}; \quad x_0/\alpha = 100; \quad y_0 = 2.25 .$$

However, the parameters required for the p_0-curve—which was run with half the amount of enzyme extract—were

$$\alpha = .8 \text{ hour}^{-1}; \quad x_0/\alpha = 96 \text{ mol cm}^{-3}; \quad y_0 = 1.52 .$$

It appears, therefore, that with the extract thus stored:

(a) During incubation a transient process takes place which is over fairly soon, certainly within 24 hours.

(b) After the termination of the transient process, the inactivations of both X and Y proceed monomolecularly, but at a much lower rate, (about $\frac{1}{4}$ and $\frac{1}{8}$ respectively for X and Y), than was the case for the fresh preparation.

(c) The rate of inactivation of X in the presence of the substrate is but slightly affected.

On the basis of available data, nothing can be said as to the nature or course of the "transient process," although we may note that for p_0

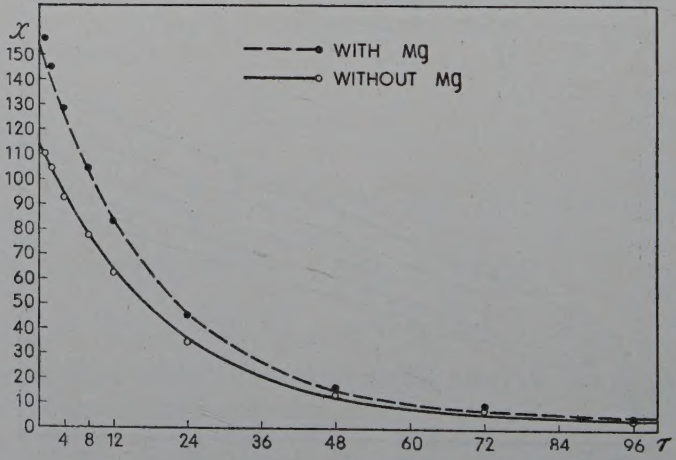

FIGURE 2

the value of x_0 is about twice, that of y_0 nearly half again the values to be expected on the basis of other curves.

After a still longer period of storage, measurements of $p_\tau(1)$ alone were made for $\tau = 0, 1, 2, 4, 8, 24, 48, 72,$ and 96 (Figure 2). From the graph and from equation (1) it follows approximately

$$p_\tau(1) = dp_\tau(0)/dt = x_\tau(0) + y_\tau(0).$$

It was found that for $\tau > 0$,

$$h = 0.0514 \text{ hour}^{-1}; \quad k = 0; \quad x_0 = 111.8; \quad y_0 = 3.2 .$$

The value of $p_0(1)$, however, instead of being 115 as would be expected from the other data, was only 94. Thus again the occurrence of a transient process during incubation is indicated, and here it is clear that this is at an end within one hour. Note that after the long period of storage, Y seems to have become completely protected from heat inactivation, whereas X breaks down even more rapidly than in the fresh preparation. From these measurements alone, α could not be determined, but the entire curve p_0 was run, from which it appeared that α had fallen to the value 0.12. Thus after the long storage, whereas X has become much more subject to inactivation by heat alone, it has become more resistant to inactivation during the reaction.

A similar set of measurements of $p_\tau(1)$ was made at the same time as that just discussed, but with Mg added ($q\ Mg = 2.3$) with the substrate (Figure 2). For this it was found that

$$h = 0.0525 \text{ hour}^{-1}; \quad k = 0; \quad x_0 = 151.2; \quad y_0 = 3.8 .$$

The difference between the two values of h cannot be regarded as significant, and one would suppose them to be the same. The effect of the Mg here seems to be to render Y slightly, X considerably more active.

Data on other extracts are considerably less complete, but serve to exhibit a definitely new feature, in that α itself is altered by incubation. Thus in equation (6), we must write everywhere α_τ for α. For one extract with incubation periods of 0, 24, and 96 hours, it was found that

$$\alpha_0 = 0.22 \text{ hour}^{-1}; \quad \alpha_{24} = 1.18 \text{ hour}^{-1}; \quad \alpha_{96} = 0.69 \text{ hour}^{-1};$$
$$y_\tau = 2.00\, e^{-0.0178\tau}; \quad x_\tau/\alpha_\tau = 66\, e^{-.0178\tau}.$$

Now, from equation (6), x/α is the amount of p produced in an infinite time by the activity of X alone, and it is this rather than x which undergoes the monomolecular decrease during incubation. It thus appears that each X molecule is limited as to the number of molecules of substrate that it is capable of decomposing, and that it is this "capacity" factor which is affected by the incubation. The α, on this interpretation, measures only indirectly the rate of breakdown of X in the presence of the substrate. Directly it measures the rate at which the X decomposes the substrate, and after the X molecule has decomposed its quota of substrate molecules, whether this be soon or late, the X molecule is capable of no further catalytic action.

This rate factor, α, is seen to vary independently of the capacity factor, first increasing and then decreasing, in a manner which cannot be further specified in the light of available data.

Returning, for the moment, to the extract previously discussed, it may be noted that since the α there was unaffected by incubation, we have no assurance that it was not actually the capacity factor of this X also which was diminished by incubation. However, in both extracts, it is clear that the Y is not so limited to any noticeable extent.

Returning now to the second extract, it was found that when Mg was added, the same loss in the capacity factor for the X was exhibited again, just as without Mg:

$$x_T/\alpha_T = 308\, e^{-0.0178T}.$$

The α's, and here also the y's, showed the same general type of variation as the α's without the Mg:

$$y_0 = 0.64 \quad ; \quad y_{24} = 2.4 \quad ; \quad y_{96} = 1.375 \quad ;$$
$$\alpha_0 = 0.0556 \text{ hour}^{-1}; \quad \alpha_{64} = 0.087 \text{ hour}^{-1}; \quad \alpha_{96} = 0.07 \text{ hour}^{-1} .$$

On a third extract, only four curves are available, for 0 and 24 hour incubation, the reaction run with and without Mg, but they show striking features. We write the equations completely:

$$p_0 = 1.76t + 114(1 - e^{-0.07t}),$$
$$p_{24} = 1.26t + 80(1 - e^{-0.1t}) ,$$

without Mg, and

$$\bar{p}_0 = 1.58t + 213(1 - e^{-0.066t}),$$
$$\bar{p}_{24} = 1.58t + 150(1 - e^{-0.047t}),$$

with Mg.

The values here obtained are extremely interesting. If we denote by a bar (e.g. $\bar{x}$) the values obtained with Mg, we note that the logarithms of the ratios of the y's, the (x/α)'s, the $(\bar{x}/\bar{\alpha})$'s, and the $\bar{\alpha}$'s are all very nearly equal to 0.014 or 0.015 times 24, indicating monomolecular breakdown with time-constant about 0.015 inverse hours. Hence the same is true of the $\bar{x}$. On the other hand

$$x_0 = \alpha_0(x_0/\alpha_0) = 7.98 \quad ; \quad x_{24} = 8;$$

and

$$\bar{y}_0 = \bar{y}_{24} = 1.58 \,.$$

Thus the heat inactivation of the Y seems to be completely reversed by Mg. As for X, it seems to be subject to several processes during incubation.

Neglecting, for the moment, the effect of Y, which is, in any case, purely additive, let us write

$$\begin{aligned} d\xi/dt &= -\lambda^{-1}\, dp/dt \,, \\ dp/dt &= \mu\, \xi \,, \end{aligned} \tag{7}$$

where ξ represents the number of moles of X, μ the number of moles of substrate decomposed in unit time by one mole of X, and λ is the number of molecules of substrate that each molecule of X is capable of decomposing. Then we find

$$p = \xi_0\, \lambda (1 - e^{-\mu t/\lambda}) \,. \tag{8}$$

Here ξ_0 is the number of moles of X present at the beginning of the reaction. If we consider the non-magnesium curves, we find that during incubation, apparently, $\xi_0\, \lambda$ decreases, μ/λ increases, $\xi_0\, \mu$ remains constant. Evidently, therefore, λ decreases, ξ_0 and μ remaining unaffected by the incubation. But from the magnesium curves, apparently, μ/λ and ξ_0 both decrease in the same way whence, presumably, λ remains constant, ξ_0 and μ both decrease.

It appears, therefore, that the modes of catalytic action of both constituents X and Y exhibited in the presence and absence of Mg are distinctly different. Thus ξ_0 and μ both show declines with, but not without Mg, while the "capacity" factor, λ, shows a decline without but not with Mg. Whatever the change undergone by X during incubation, it amounts to a limitation only of the capacity factor λ without Mg, but with Mg it diminishes both the amount available for catalysis, and the rate at which it acts.

We have been designating, in general, by X the most active, by Y the least active enzyme constituent, though without any intention of implying identity of the various X's and the various Y's. In the present instance we have not even any assurance that the X of the no-magnesium reaction might not be identical with the Y of the magnesium reaction, and vice versa. There is, in fact, a suggestion that they may be, since, as noted above, in the one case it is the x, in the other case the y which remains constant. On this assumption the X, say, which does not break down during incubation, acts rapidly but with heat-labile capacity factor in the absence of Mg, while in the presence of Mg it acts only very slowly and with a capacity factor which could not, in any case, be detected (μ being too small). On the other hand Y, which decomposes monomolecularly during incubation, acts rapidly in the presence of Mg, but at a rate μ which also decreases during incubation, whereas in the absence of Mg, its μ is too small to be detected in the exponential.

Nothing more definite than this can be said, and further speculation would hardly be profitable here. It is intended only to outline such conclusions as can be drawn from the available data, and to suggest a few possible developments. We mention, finally, one other extract upon which further work is planned. When the graph of $p_\tau(1)$ (with Mg) against τ is drawn on semi-logarithmic paper, one recognizes a curve of the following type:

$$p_\tau(1) = ae^{-a\tau} - be^{-\beta\tau} \sin \gamma \tau . \tag{9}$$

Such a curve can be obtained only if the system contains at least three constituents, and the simplest form of the equations is

$$\begin{aligned} dx/d\tau &= - Ax - Bw - Cz , \\ dw/d\tau &= x , \\ dz/d\tau &= w . \end{aligned} \tag{10}$$

Hence, assuming only a single actual catalytic element X, this X besides catalyzing the decomposition of the substrate, also "catalyzes" the development of a substance or state or condition W, which, in turn, contributes to the development of a substance, state, or condition Z. On the other hand, both W and Z act to break down the X. Nothing can be said as to the physical character of W and Z without physical or chemical measurements of a different sort. They could be any deviations of physical or chemical parameters from some "normal" equilibrium values, and as such, w and z could be either positive or negative.

The authors are indebted to Dr. H. D. Landahl for numerous helpful suggestions.

This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

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BULLETIN OF
MATHEMATICAL BIOPHYSICS
VOLUME 5, 1943

NOTE ON ANODAL EXCITATION

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A general, necessary and sufficient condition for the occurrence of anodal excitation is given on the basis of Rashevsky's two-factor theory of excitation, expressions for the anodal rheobase and the minimal shock intensity following as simple special cases.

Rashevsky's equations (Rashevsky, 1938) are equivalent to

$$\frac{d\varepsilon}{dt} = KI - k\varepsilon, \qquad \frac{dj}{dt} = MI - mj, \tag{1}$$

with excitation occurring when

$$\sigma \equiv \varepsilon - j \geqq 1, \tag{2}$$

since it is no restriction to so choose the units that the threshold ($= j_0 - \varepsilon_0$ in Rashevsky's notation) is equal to unity (cf. Young, 1941).

For brevity, we shall speak of "anodal" excitation in general as that occurring only after the cessation of the stimulus. It is a simple matter to show that if at any time when $\sigma < 1$, and either $\varepsilon > 0$, or $j > 0$, the stimulus is discontinued (cathodal break), excitation cannot ensue without further stimulation when $k > m$ (Young, 1941), but that the stimulus being discontinued when $\sigma < 1$, $\varepsilon < 0$, $j < 0$ (anodal break), excitation may ensue. If, at break or any time thereafter, the ε and j have values $\varepsilon_0 < 0$, and $j_0 < 0$, the "excitatory state" σ decays according to

$$\sigma = \varepsilon_0 e^{-kt} - j_0 e^{-mt}, \tag{3}$$

and for excitation to follow t seconds thereafter, it is necessary that at this time

$$\sigma = \varepsilon_0 e^{-kt} - j_0 e^{-mt} = 1,$$
$$\sigma' = mj_0 e^{-mt} - k\varepsilon_0 e^{-kt} \geqq 0,$$

or, what amounts to the same, that at this or *some* later time t,

$$\sigma \geqq 1, \quad \sigma' = 0. \tag{4}$$

Consider the relations between ε_0 and j_0 implied by these latter conditions. If we solve the equation (4) for t, and substitute into the inequality (4), we obtain, after elementary transformations,

$$\frac{(-j_0)^k}{(-\varepsilon_0)^m} \geqq \frac{k^k}{m^m (k-m)^{k-m}} \tag{5}$$

as the necessary and sufficient condition for anodal excitation.

In Rashevsky's theory, $k \geqq m$. To examine this condition more closely set

$$k = (1+\alpha)m, \qquad \alpha \geqq 0. \tag{6}$$

The fraction on the right then becomes

$$[(1+\alpha)^{1+\alpha} \alpha^{-\alpha}]^m,$$

and the relation may be expressed, after taking the m-th root of both sides,

$$(-j_0)^{1+\alpha} \geqq -\varepsilon_0 (1+\alpha)^{1+\alpha} \alpha^{-\alpha}. \tag{7}$$

If we further note that

$$(1+\alpha)^{1+\alpha} \alpha^{-\alpha} = (1+\alpha)(1+\alpha^{-1})^{\alpha} \geqq 1,$$

both factors approaching unity in the limit as $\alpha \to 0$, we may take the logarithm of both sides and write

$$\begin{aligned}(1+\alpha)\log(-j_0) &\geqq \log(-\varepsilon_0) \\ &\quad + [(1+\alpha)\log(1+\alpha) - \alpha\log\alpha],\end{aligned} \tag{8}$$

where the quantity in brackets is positive and, in fact, increases with α.

For convenience, let

$$y = \log(-j_0) \quad , \quad x = \log(-\varepsilon_0). \tag{9}$$

If we take the equality sign, we have a family of straight lines

$$(1+\alpha)y = x + [(1+\alpha)\log(1+\alpha) - \alpha\log\alpha], \tag{10}$$

and these lines envelope (all are tangent to) the curve

$$e^y - e^x = 1, \tag{11}$$

the point of tangency of the line defined by any α being given by

$$x = -\log\alpha = \log\frac{m}{k-m}, \quad y = \log(1+1/\alpha) = \log\frac{k}{k-m}. \tag{12}$$

The curve (11) has for its asymptotes the x-axis and the line $y = x$; it rises monotonically for all x, and has the y-intercept log 2.

The curve (11) is, of course, merely the logarithmic graph of the line

$$(-j_0) - (-\varepsilon_0) = 1 . \tag{13}$$

Hence, in terms of the ordinary graphs, we may say that the curve

$$(-j_0)^{1+\alpha} = (-\varepsilon_0)(1+\alpha)^{1+\alpha}\alpha^{-\alpha} \tag{14}$$

is tangent to the line (13) at the point given by

$$-\varepsilon_0 = m/(k-m) \quad , \quad -j_0 = k/(k-m) \tag{15}$$

and the condition for anodal excitation is that the initial point $(-\varepsilon_0)$, $(-j_0)$ shall be in the upper region bounded by the curve (14) and the line (13). In terms of the logarithmic graph, the region is defined by the line (10) and the curve (11).

The expression for the anodal rheobase is easily derived from (14) if we set therein

$$-\varepsilon_0 = K\,R_a/k \quad , \quad -j_0 = M\,R_a/m ,$$

the asymptotic values of ε and j produced by a steady current $-R_a$. We obtain the form

$$R_a = \frac{k\,m}{k-m}\,\frac{K^{m/(k-m)}}{M^{k/(k-m)}} , \tag{16}$$

somewhat different in appearance from that given by Rashevsky (1938), but easily seen to reduce to the same. In like manner, if we set

$$-\varepsilon_0 = K\,Q_a \quad , \quad -j_0 = M\,Q_a ,$$

where Q_a represents the minimal instantaneous quantity of current capable of producing anodal excitation, we obtain

$$Q_a = \frac{K^{m/(k-m)}}{M^{k/(k-m)}}\,\frac{k^{k/(k-m)}}{m^{m/(k-m)}}\,\frac{1}{k-m} . \tag{17}$$

In particular, since the minimal cathodal shock required for excitation is $(K - M)^{-1}$ (Young, 1941), this is less than Q_a if, and only if,

$$(1+A)A^{\alpha} > \alpha^{\alpha}(1+\alpha)^{-(1+\alpha)} \tag{18}$$

where

$$K = (1+A)M \quad , \quad A > 0 . \tag{19}$$

We may note, finally, that by combining a steady current with an immediately succeeding shock, ε and j can, in principle, be varied independently of one another, since with such a combination

$$\varepsilon = K(1 + kQ)/k\,,$$
$$j = M(I + mQ)/m$$

and, m being different from k, any assigned ε and j can be produced by suitable choice of I and Q. These relations, together with equation (5) or (7), make possible the testing of the theory by means of anodal stimulation over a somewhat wider range than is provided by (16) and (17) alone.

This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

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BULLETIN OF
MATHEMATICAL BIOPHYSICS
VOLUME 5, 1943

ON THE THEORY OF AMOEBOID MOVEMENTS

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It is shown how the application of the principle of maximum energy transformation, discussed in previous papers, leads to expressions which give the probability of occurrence of a pseudopod of a given size and duration in function of other physical constants of the amoeba.

In previous papers (Rashevsky, 1939; Stanton, 1943), it has been suggested that the form changes of an amoeba may be approximately treated as a quasiperiodic process of elongation and contraction. The actual irregularities due to simultaneous protrusion of several pseudopods in different directions would be considered as due to disturbing factors to be accounted for by a second approximation.

In treating quasiperiodic changes of cellular form from the point of view of the dynamics of the diffusion forces, one might think of applying the equations of deformation to each individual pseudopod in the same way as they are applied to the cell as a whole. It is, however, necessary to introduce the restraint of a constant total volume, and this makes the whole problem mathematically exceedingly difficult.

If, however, we treat the quasiperiodic changes from the point of view of the principle of maximum energy exchange (Rashevsky, 1943a, b), then the solution of the problem becomes in principle rather simple.

Let

$$N(v, l, \theta, \phi)\, dv\, dl\, d\theta\, d\phi \tag{1}$$

be the relative frequency of formation of pseudopod whose volume v, length l, duration θ and direction ϕ, as given by the angle in a plane from a fixed direction, lie correspondingly in the intervals v, $v + dv$; $l, l + dl$; $\theta, \theta + d\theta$; and $\phi, \phi + d\phi$.

If N_0 is the number of pseudopods formed during a sufficiently long time, then expression (1) represents the ratio of the number of pseudopods whose v, l, θ, and ϕ lies in the specified intervals to the total number N_0. Hence

$$\int_0^\infty \int_0^\infty \int_0^\infty \int_0^{2\pi} N(v, l, \theta, \phi)\, dv\, dl\, d\theta\, d\phi = 1. \tag{2}$$

The protrusion of a pseudopod influences the ingestion of food, and the ingestion of food constitutes the positive energy flow into the amoeba. Depending on the different assumptions which we may make about the mechanism of food ingestion, we shall arrive at different expressions for the amount of food ingested by a pseudopod characterized by given values of v, l, θ, and ϕ. In general, we could expect the amount of food ingested to increase with v, l, and θ. For instance, we may assume it to be proportional to the surface of the pseudopod. Since the crosssection area of the latter is v/l, therefore the surface will vary like $l\sqrt{v/l}$ and the amount of food ingested will vary like $\theta l\sqrt{v/l}$. Similarly a number of other assumptions may be made.

If, in general, the concentration of food is nonuniform, the expression for the amount of ingested food will involve ϕ.

For every particular assumption which we make about the process of ingestion, we can in principle determine the amount $f_1(v, l, \theta, \phi)$ of energy flowing into the amoeba during the existence of a given pseudopod.

But the protrusion of a pseudopod, by increasing the total surface, will increase the oxygen concentration in the cell. If we assume that the respiratory reactions in an amoeba are of the kind studied by H. D. Landahl (1939), then an increase in oxygen consumption will result in an increase in the oxidation rates, and hence in an increased loss of energy, due to the exothermic reactions, in which the energy is dissipated in the form of heat. By the usual approximation method, the change in oxygen concentration for a given pseudopod may be calculated. A procedure similar to that used by H. E. Stanton (1942) may be employed.

In this way we may calculate the amount $f_2(v, l, \theta)$ of the negative energy flow caused by a pseudopod. The net positive energy flow into the cell is $f = f_1 - f_2$.

We shall postulate that the distribution function (1) is such as to maximize the average total positive energy flow, due to all kinds of pseudopods.

Let us consider for simplicity, first, the case when v, l, θ, ϕ assume a discreet set of values v_i, l_k, θ_m, ϕ_r, $i, k, m, r = 1, 2, \cdots n$. Let the frequency of occurrence of v_i, l_k, θ_m, ϕ_r, be N_{ikmr}.

The total energy exchange is given by

$$\sum_{i,k,m,r} N_{ikmr} f(v_i, l_k, \theta_m, \phi_r). \tag{3}$$

If we denote by $\bar{v}$, $\bar{l}$, $\bar{\theta}$ and $\bar{\phi}$ the constant average values of the volume, length, duration and direction angle of a pseudopod, then expression (3) is to be maximized subject to the conditions

$$\sum_{ikmr} N_{ikmr}\, v_i = \overline{v}; \quad \sum_{ikmr} N_{ikmr}\, l_k = \overline{l};$$
$$\sum_{ikmr} N_{ikmr}\, \theta_m = \overline{\theta}; \quad \sum_{ikmr} N_{ikmr}\, \phi_r = \overline{\phi}. \tag{4}$$

Denoting by λ, μ, ν, and ω Lagrangian multipliers, the above requirement gives

$$f(v_i, l_k, \theta_m, \phi_r) + \lambda v_i + \mu l_k + \nu\theta_m + \omega\phi_r = 0. \tag{5}$$

The N_{ikmr}'s drop out entirely. Hence, with those assumptions the frequencies of occurrence of the different pseudopods are not intrinsically determined. They may be assumed to be due to pure chance, and $N(v, l, \theta)$ would be a normal distribution, independent of ϕ.

Equation (5), which for the continuous case becomes

$$f(v, l, \theta, \phi) + \lambda v + \mu l + \nu\theta + \omega\phi = 0, \tag{6}$$

can in principle be verified experimentally, since it holds for each pseudopod, and the parameters λ, μ, ν and ω can be determined from observation.

Though the distribution may not depend explicitly on ϕ, yet that quantity affects the situation through relation (6). While a pseudopod in any direction may be equally probable *a priori*, yet for the pseudopods in different directions, the relations between v, l and θ will be different, for they will correspond to different values of ϕ, which now plays the role of a parameter. As remarked before, the dependence of ϕ will enter only through the nonhomogeneity of the external medium. The equal probability for pseudopods in any direction would result in a zero average displacement of the amoeba as a whole. This might be the case for an absolutely homogeneous external medium, for the amoeba moves towards regions of higher food concentrations.

We may, however, consider a more general case in which the distribution function is itself intrinsically determined by the requirements of a maximum energy interchange.

When a pseudopod is protruded, this increases the positive energy exchange because of increased food ingestion. Remembering that an amoeba ingests rather large particles, the distribution of which in the medium is not necessarily uniform, we see that if a pseudopod of the same size, shape and direction is protruded too frequently, the average increase of food ingestion per protrusion will decrease because of exhaustion of food in that region. Hence the function f_1 will depend not only on v, l, θ and ϕ, but also on $N(v, l, \theta, \phi)$. Considering again the case of discrete values, we have

$$f_1 = f_1(v_i, l_k, \theta_m, \phi_r, N_{ikmr}). \tag{7}$$

Instead of expression (3) we now must maximize the expression

$$\sum_{ikmr} N_{ikmr} [f_1(v_i, l_k, \theta_m, \phi_r, N_{ikmr}) - f_2(v_i, l_k, \theta_m)], \tag{8}$$

subject to conditions (4). We now find

$$\begin{gathered} f_1(v_i, l_k, \theta_m, \phi_r, N_{ikmr}) - f_2(v_i, l_k, \theta_m) + N_{ikmr}\frac{\partial f_1}{\partial N_{ikmr}} \\ + \lambda v_i + \mu l_k + \nu\theta_m + \omega\phi_r = 0 . \end{gathered} \tag{9}$$

This is an algebraic equation in N_{ikmr}, which determines the latter in function of v_i, l_k, θ_m, ϕ_r, λ, μ, ν and ω; thus giving for the continuous case the function $N(v, l, \theta, \phi, \lambda, \mu, \nu, \omega)$. The parameters λ, μ, ν and ω are determined from equations (4), which for the continuous case become:

$$\int \cdots \int vN(v, l, \theta, \phi, \lambda, \mu, \nu, \omega)\, dv\, dl\, d\theta\, d\phi = \overline{v} \tag{10}$$

and three corresponding equations for l, θ and ϕ. We have four equations for the determination of the four quantities λ, μ, ν and ω.

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BULLETIN OF
MATHEMATICAL BIOPHYSICS
VOLUME 5, 1943

MATHEMATICAL BIOPHYSICS OF CELL DIVISION

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The new fundamental equation, based on the principle of maximum energy exchange, is applied to the case of elongation and constriction of a cell. A simple case is treated as an illustration, and it is shown that the elongation curves thus obtained are of the same character as in the old theory.

Presupposing familiarity of the reader with previous publications (Rashevsky, 1943 a, b, c, d; hereinafter referred to as [a], [b], [c], [d]), we shall apply the fundamental equation of [c]:

$$f_i \frac{dx_i}{dt} = \tau \frac{\partial F}{\partial x_i} - \frac{\partial H}{\partial x_i} \tag{1}$$

to the problem of elongation and constriction of dividing cell.

Neglecting the energy dissipation in the diffusion field itself [d], the expression for F in a cell is some function of the concentration $\bar{c}_k$ of the different metabolites. Those $\bar{c}_k$ are in their turn determined by the different diffusion coefficients, permeabilities, reaction rates, as well as by the two coordinates, which roughly describe the shape of the cell, namely its length $2r_1$ and the width of the neck r, ([b], [d]). The function $F(r_1, r)$ is determined in any particular case by the approximate solution of the diffusion equation.

Considerations of viscous flow determine the quantities f_{r_1} and f_r which give us the viscous forces $f_{r_1}(dr_1/dt)$ and $f_r(dr/dt)$ of resistance to the elongation and constriction. H is given by the potential energy of the surface tension. If S denotes the surface of the cell, then

$$H = \gamma S. \tag{2}$$

The question may be raised as to whether the potential energy due to the diffusion forces should also be included into H. Although the diffusion forces are formally derivable from a potential, yet their very nature is based on non-conservative phenomena, and therefore such energy considerations as have been used before (Rashevsky, 1938, Chapter viii) have always been felt as somewhat uncertain. There is also this fundamental limitation of the diffusion forces: their effects vanish with infinite D_e. This is extremely implausible biologically, and has been always the weak point of the theory. In cases of protoplasmic streamings accompanying division, there remains the old difficulty; namely, although the streaming itself is rather an indication of the existence of the forces, their distribution may be upset considerably by the streaming, so as to strongly modify their effects. It is more likely therefore that the diffusion forces should not be *generally* included into H, although they might be included in special cases. We shall therefore omit them here. Other sources of potential energy, such as possible elastic forces should, however, when present, be included in H.

The system of differential equations which describe now the elongation and constriction is:

$$\begin{aligned} f_{r_1}\frac{dr_1}{dt} &= \tau\frac{\partial F(r_1,r)}{\partial r_1} - \gamma\frac{\partial S(r_1,r)}{\partial r_1}; \\ f_r\frac{dr}{dt} &= \tau\frac{\partial F(r_1,r)}{\partial r} - \gamma\frac{\partial S(r_1,r)}{\partial r}. \end{aligned} \tag{3}$$

As an illustration merely, we shall apply equations (3) to the simple idealized case when a cell elongates without constriction, so that the second equation drops out, and when we have only one substance, produced exothermically at a rate proportional to its concentration. We shall also for simplicity put $D_e = h = \infty$, and denote D_i by D.

The frictional force may be computed by considering the elongating cell as a plastic rod of length r_1 and width r_2. For such a rod we have

$$\frac{1}{r_1}\frac{dr_1}{dt} = \frac{1}{3\eta}\frac{P}{s}, \tag{4}$$

where P is the total *force,* and s the crossection.

Remembering that

$$s = \pi r_2^2; \quad \frac{4}{3}\pi r_1 r_2^2 = \frac{4}{3}\pi r_0^3 = V; \quad r_1 r_2^2 = r_0^3; \tag{5}$$

solving equation (4) for P and eliminating r_2 by means of (5), we find for the force the expression

$$f_{r_1}\frac{dr_1}{dt}=\frac{9\eta V}{4r_1^2}\frac{dr_1}{dt}. \tag{6}$$

Denoting by w the heat of reaction per gram of substance produced, and remembering that the reaction is exothermic, we have, denoting the rate of reaction by $a\bar{c}$:

$$F=-Vwa\bar{c}\,. \tag{7}$$

But

$$\bar{c}=\frac{c_0}{1-a\Lambda};\quad \Lambda=\frac{r_1^2\, r_2^2}{3D(2r_1^2+r_2^2)}. \tag{8}$$

Hence, eliminating r_2 by means of expression (5), we have

$$F=-3VDwac_0\frac{2r_1^2+r_0^3/r_1}{3D(2r_1^2+\frac{r_0^3}{r_1})-ar_0^3\, r_1}, \tag{9}$$

and therefore

$$\frac{dF}{dr_1}=\frac{9V^2Dwa^2c_0}{2\pi}\,\frac{r_1^2-r_0^3/r_1}{[3D(2r_1^2+\frac{r_0^3}{r_1})-ar_0^3\, r_1]^2}. \tag{10}$$

For $r_1>r_0$, $dF/dr_1>0$. It is zero for $r_1=r_0$ and for $r_1=\infty$.

Introducing (6), (10) and (2) into the first equation (3) we have after rearrangements:

$$\frac{dr_1}{dt}=\frac{2VDwa^2c_0\tau}{\pi\eta}\,\frac{(r_1^3-r_0^3)r_1}{[3D(2r_1^2+\frac{r_0^3}{r_1})-ar_0^3\, r_1]^2}-\frac{4r_1^2\gamma}{9V\eta}\frac{dS}{dr_1}. \tag{11}$$

The last term in equation (11) may be calculated by approximating the cell by an ellipsoid. The general expression is rather cumbersome. For small values of elongation r_1-r_0, we have (Landahl, 1942)

$$S=4\pi r_0^2+1\cdot 6\,\pi r_0^2\left(\frac{r_1}{r_0}-1\right)^2 \tag{12}$$

and therefore

$$\frac{dS}{dr_1}=1\cdot 6\,\pi(r_1-r_0)\,. \tag{13}$$

Since for very small values of r_1-r_0 the first term of the right side of equation (11) may be written

$$\frac{6VDwa^2 c_0 r_0^3 \tau}{\pi\eta(9Dr_0^2 - ar_0^4)^2}(r_1 - r_0),$$

the requirement that $dr_1/dt > 0$ for $r_1 = r_0$ leads to, remembering equation (13),

$$\frac{8Dwa^2 c_0 r_0^6 \tau}{(9Dr_0^2 - ar_0^4)^2} > \frac{1 \cdot 6\,\gamma}{3\,r_0} \tag{14}$$

which is satisfied only when r_0 exceeds a critical radius, determined by making the two sides of expression (14) equal. The universal constant τ is one of the factors determining the critical radius.

For very large values of $r_1 - r_0$ considering the cell as a very long cylinder, the surfaces of whose ends can be neglected, we have $S = 2\pi r_0^{3/2}\sqrt{r_1}$ and therefore, $dS/dr_1 = 2\pi r_0^{3/2}/r_1^{1/2}$. The last term of equation (11) now becomes $(2\gamma/3\eta)(r_1^{3/2}/r_0^{3/2})$. Since the first term of the right side of equation (11) tends to a constant value for large values of $r_1 - r_0$, therefore when a certain value of r_1 is exceeded, dr_1/dt becomes zero, even though inequality (14) is satisfied. Hence the general shape of the elongation curve is the same as in the old theory. Elongation however occurs even for $D_e = h = \infty$. It occurs also for $D_i = D_e = \infty$, h—finite, when no volume diffusion forces can be present.

From the comparison of calculated and observed elongation curves we may determine the constant τ. It can also be determined in principle from the critical radii, although this would not be practical.

The author is indebted to Dr. Herbert D. Landahl for verifying the calculations.

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BULLETIN OF
MATHEMATICAL BIOPHYSICS
VOLUME 5, 1943

STUDIES IN THE MATHEMATICAL BIOPHYSICS OF DISCRIMINATION AND CONDITIONING: III

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The theory dealing with recognition learning is generalized to include five variables. These are the errors, trials, possible number of choices, the number of items and the fraction of prompting. The theory is then extended to include recall learning. This extension necessitates the introduction of a variable which is the number of trials without responses, i.e., the third category of judgment. The strengths of reward and punishment are also introduced as variables. Thus a curve in nine dimensions is determined.

In previous studies we have considered a neural mechanism which exhibits properties of discrimination and conditioning (Landahl, 1941a, b, hereinafter referred to as I and II respectively). It is our purpose here to generalize the previous results for the case of recognition learning as well as to introduce recall learning. We presuppose familiarity of the reader with the previous two papers.

1. *Recognition learning.* We consider first the situation in which recognition of the learned response is all that is required. The experimental situation to be considered is much the same as that described in II. Let there be M pairs of stimuli whose association is to be learned. Let one of each pair, $S_i (i = 1, \cdots M)$ be the key stimulus which when presented will, after sufficient learning, illicit the response normally given to the other stimulus of the pair. This latter, $S'_j (j = 1, \cdots N)$, is one of a group of N stimuli arbitrarily chosen, except that the group includes the stimulus to be associated with the key stimulus. When a stimulus S_i is presented, the subject is to choose one of the S'_j stimuli. Thus he has N possible choices. We assume that enough time is allowed so that a choice is made on each trial n. If the choice is correct, the subject is so informed or rewarded, and this contributes to c_i, the cumulative correct responses when S_i is presented. As we consider the M stimuli S_i to be of the same intensity we may drop the subscript. Thus c is to be identified with the average of the experimental c values. If the choice is wrong, it contributes to the cumulative errors, w. The subject is then informed of the error or punished. He may or may not be shown the correct response subsequently. In general, the experimenter may tell the subject as to

which is the correct response a certain fraction $(1 - f)$ of the times in a particular experiment. If $f = 1$, there is no prompting, and if $f = 0$, prompting follows each trial. Thus the variable f must be on the closed interval from 0 to 1.

We consider that the neural mechanism has essentially the properties discussed in I and II. We assume that it is sufficiently complex so that conditioning may take place between any S_i and S'_j. The conditioning may be such that the tendency for S_i to produce the response to S'_j is either strengthened or weakened. For simplicity we shall neglect any bias so that at the first trial the N stimuli, S'_j, are equivalent. Let ε_0 be the initial value of the excitation factor corresponding to any one of the N stimuli. After c correct responses to the S'_j stimulus associated with one particular S_i stimulus, the effective value of the excitation factor for the correct stimulus will have increased to some value ε_c due to the conditioning. The $N - 1$ wrong responses will each have decreased by various amounts. But, as in II, we shall not consider the individual wrong responses, only their total. Thus the average value of the excitation factor for the $N - 1$ wrong stimuli is all we shall use, and it is given by some value $\bar{\varepsilon}_w$ after w wrong responses have been made.

As only one of the S'_j stimuli can be chosen, we again consider that there are cross inhibitory fibers which tend to block out all but the stimulus which produces the strongest excitation. Introducing the threshold fluctuations, we shall expect each stimulus S'_j to have a certain probability P_j of being the stimulus to pass through, and this probability will be greater for the stronger excitations. In general, this relation will depend upon each of the stimuli, upon the conditioning which has taken place in all possible combinations, and upon the number N. We shall, however, use the approximate expression (I, eq. 9)

$$\log \frac{NP_w}{N-1} + k(\varepsilon_c - \bar{\varepsilon}_w) = 0, \tag{1}$$

where k is a constant and P_w is the probability of a wrong response at n trials. There being only two categories of judgment, the probability of a correct response, P_c, is equal to $1 - P_w$.

Considering again that the strength of conditioning varies linearly with the number of correct responses, we will have for ε_c an amount $bc + b(1 - f)w$ added to ε_0, where b is a constant. In general we might have introduced two constants, as the guessed response and the response due to prompting are not identical. The mechanism does not allow this distinction without further complications. We shall thus consider only the simpler case. However, it may be pointed out that by permitting f to have values somewhat different from their preassigned values, this effect can be included. Thus, if f must be assigned a value f' instead of f, we would interpret the discrepancy to mean that the ratio of the actual coefficient of $(1 - f)w$ in (2) is given by $(1 - \mathrm{f})/(1 - f')$. This ratio could then be introduced as an additional parameter. Subsequently we shall introduce factors which will complicate such an interpretation, so that the above would only be an approximation.

The act of responding to any stimulus, together with the subsequent punishment or reward will, in general, react on the state of the conditioning at any center. In terms of the neural mechanism, this means that inhibitory fibers leading to various conditioning centers may be excited, thus tending to disrupt the conditioning. After n trials, there will have been Mc correct responses altogether. Thus an amount roughly proportional to $(M - 1)c$ is subtracted from the conditioning at a center corresponding to a particular stimulus S_i. Without loss of generality, we let this amount be proportional to Mc. In general, one would expect that the inhibitory effect would increase with Mc at a decreasing rate. Making such an assumption increases the complexity considerably. However, if instead of M, we write a function of M which increases at a decreasing rate, no great difficulty is introduced. Thus we may write for the amount subtracted; $\zeta(M)c$, which to a first approximation may be written $\zeta' Mc$, or to a better degree of approximation $\zeta'(1 - e^{-\mu M})c/\mu$, ζ being a suitable function of M, and ζ' and μ being constants. In a similar manner we must subtract a quantity $\eta(M)w$ for the effect of the wrong responses, $\eta(M)$ being similar to $\zeta(M)$. Furthermore, each S'_j stimulus is itself responded to in some way, although not recorded by the observer. Thus one should subtract a quantity $\theta(MN)n$ as this type of response takes place MN times at each trial. $\theta(MN)$ is a function similar to $\zeta(M)$, but probably very much smaller in value. On adding together the various terms algebraically, we may obtain an expression for ε_c.

An expression for $\bar{\varepsilon}_w$ can be obtained analogously. The principal term in the contribution to $\bar{\varepsilon}_w$ due to the conditioning to avoid wrong responses is $\beta\, w/(N - 1)$, where β is a constant analogous to b. For each wrong response we have an amount β at one of the centers corresponding to one of the $(N-1)$ wrong responses. Thus an amount $\beta/(N - 1)$ is contributed to the average for each of the w wrong responses. This result may also be obtained if we recall that learning to eliminate an error is $1/(N - 1)$ times as effective as learning a correct response. Similarly, each of the other terms one should add would be divided by $(N - 1)$. Unless the effect of punishment is much greater than the effect of reward, we may then neglect additional terms, leaving $\bar{\varepsilon}_w = \varepsilon_0 - \beta w/(N-1)$.

Subtracting the expression for $\bar{\varepsilon}_w$ from the expression for ε_c, we obtain

$$\varepsilon_c - \bar{\varepsilon}_w = (\gamma n - \delta w)/k, \tag{2}$$

where

$$\gamma = kb - k\zeta(M) - k\theta(MN), \tag{3}$$

$$\delta = kbf - k\beta/(N-1) - kf\zeta(M) + k\eta(M). \tag{4}$$

Substituting (2) into (1), and dw/dn for P_w and eliminating c by $c = n - w$, we obtain a differential equation in w and n. Integrating this equation, we have

$$w = \frac{1}{\delta} \log \frac{N\gamma}{(N-1)\,\delta\, e^{-\gamma n} - (N-1)\,\delta + N\gamma}. \tag{5}$$

Equation (5), with γ and δ replaced by their equivalents in (3) and (4), determines a surface in a five dimensional space defined by the variables w, n, f, M, and N. In some experimental situations, one could, perhaps, neglect the terms in β, θ, and η, as well as the μ in $\zeta(M)$, thereby determining very roughly the surface by two parameters. This is essentially what was done in II for the special case in which $f = 1$, $M = N$. The term in θ would be expected to be small so that $\theta' MN$ should be ample. The term in η could be similarly treated unless the punishment is considerable. Even if so, one could perhaps introduce the same μ as in $\zeta(M)$. The result would still be only six parameters for the determination of a surface in five dimensions. Note that k multiplies each of the parameters in (3) and (4), and thus is not an additional parameter.

In order that c and w remain positive and real, and that d^2c/dn^2 be positive for $n > 0$, it is necessary and sufficient that

$$\phi \equiv \gamma - \delta + \delta/N \geqq 0. \tag{6}$$

This is the requirement necessary for any learning at all. If also $\gamma \geqq 0$ (but not both equality signs holding simultaneously), then $w \rightarrow w_\infty > 0$ for $n \rightarrow \infty$, i.e. learning is complete. But if $\gamma - \delta + \delta/N > 0$ and $\gamma < 0$, then $w \rightarrow \infty$ and $dw/dn < 1$ for $n \rightarrow \infty$, that is, the learning is incomplete. The condition that there be an interval in M, the number of items to be learned, in which learning is incomplete is

$$\zeta'[\beta - (N-1)\, f\, \theta'] > (N-1)\, \eta'(b - \theta'), \tag{7}$$

provided that a positive, real M satisfies $\phi = 0$ and if we set $\theta(MN) = \theta' MN$. From inequality (7) we find that in order to have any "incomplete" learning, θ and N must be small enough. Furthermore, if

(7) is satisfied for $f = 0$, it may no longer be when $f = 1$. That is, the interval for incomplete learning is diminished when prompting is reduced, and this is especially effective when N is increased.

The value of $N(dP_c/dn)/(N - 1)$ for $n = 0$,

$$N\, d^2c/dn^2/(N - 1) = \phi\,, \tag{8}$$

gives the rate of change of the probability of a correct response corrected for chance as a function of the number of trials, and is a measure of the rate of learning. This measure decreases monotonically with both M and N.

The parameters b and ζ' will increase with the strength of the reward. If the increase is roughly proportional, and if the increase in b is greater, the rate of learning will increase with the strength of the reward. If these relations are not proportional, the dependence of the rate of learning on reward is more complex. The parameters β and η will depend on the strength of the punishment. If both are proportional, the rate of learning will increase with increased punishment for small enough N, but will decrease for large enough N. That is, in a sufficiently complex situation, increasing punishment may be detrimental to the learning. If the β and η are not proportional to the amount of punishment, the dependence of the learning upon the latter is more complex.

2. *Recall learning.* Thus far we have considered only recognition learning. When we treat the case of recall learning, we simply remove the ε_0 values corresponding to the S'_j stimuli since they are no longer presented. With successive trials, however, these stimuli are learned, that is, there develops an ε'_0 which plays the same role as ε_0, tending to produce the response which is normally illicited by the particular S'_j stimulus. This ε value is of the form $[b_1 - \zeta_1(M)]n$, the stimulus being presented on each trial. Clearly we cannot require a response each trial, and thus we must be dealing with the case of three categories of judgment, the intermediate category being failure to respond. In terms of the mechanism, this means that there is a threshold h which $\varepsilon_c - \varepsilon_w$ must exceed for a correct response to be given. This quantity h is not a constant in general (Landahl, 1939). We shall postulate that h decreases only because of the increase in the ε'_0 values. In general, h would decrease to some positive limit which might be zero. Unless this expression is linear, considerable difficulties arise in obtaining solutions. Thus we shall set

$$h = h_0 - b_1 n - \zeta_1(M)n = h_0 - \gamma_1 n/k \tag{9}$$

when the right member is positive, otherwise $h = h_1$. In order to further simplify the results and avoid introducing an additional parameter, we shall take $h_1 = 0$.

For ε_c we have an expression similar to that of the previous section, except that $f = 0$, and that ε_0 and the term in N vanishes, that is $\theta = 0$. But it should be noted that the terms in w arise only where errors are made. Let w_0 be the accumulated failures to respond. An error is defined as a response to one of the M stimuli S_i which is not the correct response. If any other response is given, it is counted as a failure to respond. Thus we set $M = N$.

For $\bar{\varepsilon}_w$ we have a term $\beta w/(M - 1)$ multiplied by some function of n which is small initially but approaches unity. We shall, however, neglect this variation and take $\bar{\varepsilon}_w$ to be equal to $\beta w/(M - 1)$. If β turned out to be somewhat smaller in this case as compared with that discussed above, this would be expected. One might introduce a factor of the order of one half in this term with justification.

We continue to use the approximation of the form of (1), except that now we have the three category case. Thus instead of (1), we have (cf. Landahl, 1939),

$$\log \frac{MP_w}{M-1} + k(\varepsilon_c - \bar{\varepsilon}_w + h) = 0, \tag{10}$$

$$\log \frac{M(1-P_c)}{M-1} + k(\varepsilon_c - \bar{\varepsilon}_w - h) = 0. \tag{11}$$

These expressions are defined for all values for which the term in parentheses multiplied by k are positive. Since $\varepsilon_c - \bar{\varepsilon}_w$ and h are always positive, the first expression is defined for all values under consideration. The second expression, however, breaks down when $\varepsilon_c - \bar{\varepsilon}_w - h < 0$, since for large enough negative values of this term, P_c as defined will exceed unity. This difficulty can be avoided by introducing a new definition for P_c in which continuity in P_c and in the first derivative of P_c with respect to $(\varepsilon_c - \bar{\varepsilon}_w)$ is required, as well as that P_c should have a lower bound of zero. If an exponential type of curve is again used, these conditions lead to

$$\log MP_c + (M-1)k(h - \varepsilon_c + \bar{\varepsilon}_w) = 0 \tag{12}$$

for $h - \varepsilon_c + \bar{\varepsilon}_w > 0$.

The value of $\varepsilon_c - \bar{\varepsilon}_w$ is given by $\gamma n - \delta w$, γ and δ being given by (3) and (4) but with $\theta = f = 0$. Now let n_1 be the solution of $h - \varepsilon_c + \bar{\varepsilon}_w = 0$ or

$$h_0 - \gamma n_1 + \delta w(n_1) - \gamma_1 n_1 = 0, \tag{13}$$

γ_1 being the coefficient of n in (9), $w(n_1)$ being determined from

(10). We shall solve only for P_c and P_w, that is, dc/dn and dw/dn. For $n \leqq h_0/\gamma_1$ we have from (9) and (10)

$$P_w = dw/dn = \frac{(M-1)\, e^{(\gamma_1-\gamma)n}(\gamma_1-\gamma)}{M(\gamma_1-\gamma)e^{kh_0} - (M-1)\,\delta\,(e^{(\gamma_1-\gamma)n}-1)}. \qquad (14)$$

For $n \leqq n_1$ we have from (12), eliminating w by the solution of (14)

$$P_c = dc/dn = \frac{e^{-(M-1)kh_0}}{M}$$
$$\times \left[\frac{M(\gamma_1-\gamma) - (M-1)\,\delta\, e^{-kh_0}(e^{(\gamma_1-\gamma)n}-1)}{M(\gamma_1-\gamma)}\right]^{M-1} e^{(M-1)(\gamma_1+\gamma)n}, \qquad (15)$$

while for $n_1 \leqq n \leqq h_0/\gamma_1$, we have from (11), again eliminating w by (14),

$$P_c = 1 - \frac{(M-1)(\gamma_1-\gamma)\, e^{-(\gamma_1+\gamma)n}\, e^{kh_0}}{M(\gamma_1-\gamma)-(M-1)\,e^{-kh_0}\,\delta\,(e^{(\gamma_1-\gamma)n}-1)}. \qquad (16)$$

For $n \geqq h_0/\gamma_1$, we have

$$\begin{aligned} P_w = 1 - P_c = {} & [(M-1)\,\gamma\, e^{-\gamma n}]/[M\gamma - (M-1)\,\delta\,\gamma \\ & \times e^{-kh_0}(e^{(\gamma_1-\gamma)h_0/\gamma}-1)/(\gamma_1-\gamma) \\ & - (M-1)\,\delta\,(e^{-\gamma h_0/\gamma_1} - e^{-\gamma n})]. \end{aligned} \qquad (17)$$

Throughout, dw_0/dn is obtained from $dw_0/dn = 1 - P_c - P_w$.

Equations (14) to (17) define a surface in terms of the variables w, c, n and M, w_0 being given by $n - c - w$. Three new parameters have been introduced, h_0 b' and ζ'; b and ζ can be determined from an experiment corresponding to requirements discussed above. But a new variable w_0, has been introduced, as well as a new experimental situation which is equivalent to another variable.

It should be noted that in the case of recognition learning, we did not consider the possibility of failures to respond. It is possible to introduce a threshold h in that case also. However, in such experiments, ample time is generally given for some response to occur. Furthermore, the motivation to make a response each time is fairly strong. It would thus be necessary to set a time limit for each trial to make the experimental situation correspond with the case in which an h is introduced. But in the case of recall learning, it is clear that for the first few trials, there will be no wrong or correct responses. Thus we see that the relationship between recognition and recall learning has something in common with the relationship between the two and three category cases in discrimination.

Although not explicitly introduced thus far, we have two additional variables. These are the strength of the reward, r, and the strength of the punishment ρ. In some situations these can be given rough measures. To a first approximation one might set b , b_1 , ζ and ζ_1 proportional to r and β and η proportional to ρ . We may also introduce explicitly a variable D which is zero for the case of recognition learning and one for recall. The values of w can be obtained for $D = 0$ and $D = 1$. If these are w' and w'', we may set $w = (1 - D) w' + D w''$, thereby combining both cases into a single expression. Similarly, instead of w_0 we may write $D w_0$ so that it vanishes for $D = 0$ and equals w_0 for $D = 1$. The dependent variable c may be given by $n - w - w_0$, w and w_0 being the new variables. We thus have the two dependent variables w and w_0 determined as functions of the seven independent variables n , M , N , f , D , r and ρ.

For a preliminary investigation one might set $\theta = 0$, and introduce the same coefficient μ in the expressions $\zeta(M)$, $\eta(M)$, and $\zeta_1(M)$, although in ζ_1 and perhaps also in η , one might set $\mu = 0$. It would also seem plausible to set $b/\zeta = b_1/\zeta_1$. Thus if $b = a_1 r$, $\zeta' = a_2 r$, $b_1 = a_3 r$, $\zeta'_1 = a_3 a_2 r / a_1$, $\beta = a_4 \rho$, $\eta' = a_5 \rho$, $\mu = a_6$ and $h_0 = a_7$, we shall require seven parameters, a_1 , $a_2 \cdots a_7$, to determine a curve in a nine dimensional space.

This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

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BULLETIN OF
MATHEMATICAL BIOPHYSICS
VOLUME 5, 1943

BOOK REVIEW

D'ARCY WENTWORTH THOMPSON. *On Growth and Form.* Second Edition, 1942. II + 1116 pp. Cambridge: At the University Press; New York: The Macmillan Company. $12.50.

The new edition follows its predecessor after 25 years and has grown from 729 to 1097 pages. Two chapters, namely III and VII, have doubled in volume, while others exhibit a correspondingly lower rate of relative growth.

The purpose of the book is stated on page 15: "We want to see . . . how the form of living things . . . can be explained by physical considerations," and in the Epilogue the author writes: "My task is finished if I have been able to show that a certain mathematical aspect of morphology is essential to (the) proper study of Growth and Form. . . . For the harmony of the world is manifest in Form and Number." The term "Form and Number" is used once more, and the Epilogue ends with a eulogy of Henri Fabre who "saw in Number le comment et le pourquois des choses." This change of "Growth and Form" into "Form and Number" and finally into "Number" appears, to the reviewer at least, as significant. For the book does not deal so much with relation between Form and Growth, as with Growth and with Form. The bond between the two is frequently no more than a formal one, namely, the mathematical treatment of the subject.

Thompson's conception of form is of classical simplicity. "When we abstract . . . from the material to the form, then force is the appropriate term for . . . the causes by which these forms . . . are brought about." Sir Oliver Lodge is cited: "You can move matter, it is the only thing that you can do to it."

The principles according to which Form is explained vary. Chapter V contains, apropos of surface tension and its influence on Form, an explanation of the method of maxima and minima and the principle of least action. Another viewpoint is developed in the discussion of the equiangular spiral: "The evidence of (a) constant relativity of Growth is of the essense of the equiangular spiral." It is here that form is directly considered as a result of growth.

Growth forms the subject matter of Chapter III, entitled "The Rate of Growth." It occupies itself mostly with ontogenetical problems. In discussing brain weight and body weight, however, there appears a table illustrating that "the ratio of magnitude tends to change not only as the individual grows but also with change of bodily size from . . . one species to another," and near the very end of Chapter III we find the statement that "changes of form are essentially the same . . . whether they be episodes in the life history of the individual, or mainfest themselves as the distinctive characteristics of the species of the race," (the word race is here evidently intended to denote genus, not a subdivision of a species). "From one form to another there is but one road of transformation, and . . . it will proceed in the self-same way whether it occurs within the lifetime of an individual or during the long ancestral history of a race."

This shift from ontogenesis to phylogenesis is completed in the last, and probably most famous chapter, "On Transformation." The principle is well known:

Of two (or several) forms to be compared, one is inscribed into rectangular Cartesian coordinates. It is required to find the deformation (or deformations) these coordinates have to undergo in order to reproduce the other form (or forms). The chapter starts out with simple examples, but leads up to problems requiring curvilinear deformations. No attempt is made to handle these problems analytically. The diagrams are startling and fascinating, and it is all the more a matter of surprise that they have been comparatively little used by other authors. It still remains to be seen whether an improved technique could render the method more useful. The method obviously represents problems of conformal representations. Thompson cites a paper by Ikada and Kuwaori on conformal representation by means of elliptic integrals. A powerful tool could perhaps be forged out of the methods of these Japanese authors, which, written from the standpoint of a physicist, are not immediately applicable to Thompson's problem.

Thompson is essentially a biologist who argues from the concrete instance to the general. This bent of mind is well illustrated by his discussion of cell division. It is interesting to compare Thompson's treatment with that of Rashevsky, Landahl and Young, which is given but scant justice in the book. Rashevsky starts out from the simplest assumption of the cell as a metabolizing system. Thompson watches under his microscope how a cell divides and gets immediately involved in karyokinetic spindles. He does not get beyond a vague analogy with magnetic forces, culminating in the statement (in italics): "A body placed in the medium will tend to move towards regions of greater or lesser force according as its permeability is greater or less than that of the surrounding medium."

The book contains a prodigious wealth of information.. Written in a broad style and with frequent digressions from the main topic, it has, for these very reasons, a great and unique charm. On opening it at almost any place, one feels immediately that warmth which only civilized and generous persons are able to impart. And Thompson's erudition goes far beyond the confines of biology. References to the older literature are frequent, and philosophers and mathematicians are cited freely, be it in English, French, German or ancient Greek.

The forms of animals and plants are universally explained on the basis of organic evolution. What is the author's attitude toward this concept? On page 11 we read: "Organic evolution has its physical analogue in the universal law that the world tends to pass from certain less probable to certain more probable states . . . " and a few lines further, this is described as the law of entropy. While Thompson states on page 14 that he is not concerned with the "weighty problem" of explaining "the long battle against the cold and darkness which is death," he nevertheless appears to have opened the door to misunderstanding.

Progress in organic evolution has been defined as increased independence of, and control over, the environment. This clearly makes evolution dependent of the environment which in its turn must be thought of as a function of entropy. In the light of the definition just cited, organic evolution would be rather dependent upon, than an analogue of, the law of entropy. To reconcile entropy and organic evolution is one of the most difficult problems of biology. Entropy is defined as probability and the law of entropy states that the final state of the world is probably predetermined. Does the analogy assumed by Thompson imply that, to cite but one concrete instance, homo sapiens is a more probable state of matter than a unicellular organism? There are, of course, many fewer men than there are microorganisms on this earth, therefore homo sapiens is less probable in the strictly statistical sense of that term. There is, as far as we can see, no difference in the probability of man and of amoeba in a thermodynamical sense.

It will readily be admitted, however, that the human organism is more complex than amphioxus or an amoeba. Complexity may be hard to define. The number of motor patterns which an organism is capable of achieving may afford an objective measure of complexity. Thus fishes have but few motor patterns: locomotion, steering, breathing, mastication, eye movements probably complete their inventory. Man, on the other hand, can move his head, his hands and his arms independently from the rest of his body, can use his larynx for articulate speech and his facial musculature to express his emotions. Now if it is admitted that complexity is a less probable state for (living or dead) matter than simplicity, then it follows that organic evolution and the law of entropy are not analogues but opposites. "Life resembles the sailor who climbs up the mast of a sinking ship," is the gist of a remark that the reviewer read years ago but failed to track down again. If organic evolution were a case of entropy, the battle against cold and darkness could never have been waged.

In an addition to the second edition, the author cites Kant who states that the "axiom of casuality is *denknotwendig*, for experience itself depends upon its truth." (p. 21). Elsewhere, (p. 268 et seq.) Thompson hints that "the great function of natural selection is not to originate but to remove," and adds that "the world of things living . . . pursues its ceaseless course of creative evolution." One is reminded of a passage of Whitehead's "Adventures of Ideas" (p. 368/369): "Decay, Transition, Loss, Displacement belong to the essence of the Creative Advance." But Thompson is far from Whitehead, whose name does not even appear in the index. As we just saw, Thompson follows Kant in his conception of the law of causality. What, then, is meant by *creative* evolution? Clearly, creativity implies the negation of an unrestricted reign of causality, for it presupposes spontaneity. It is interesting to see here how Thompson, though deeply steeped in mechanism, has still not completely avoided the terminology of an entirely incompatible position.

Furthermore, the connection between ontogenetic and phylogenetic processes appears in practice to be far from "easy and legitimate", as the author states. The problem of brain weight and body weight, adduced by the author, is a case in point. It is easy to find a comparatively simple functional relation between these two variables when the universe of discourse is defined, let us say, to be the class of adult mammals. But when we deal with the class of all developmental stages of a mammal, it is not possible to find a simple functional relationship, since, as Scammon has shown, body-weight follows another type of growth than brain weight. Since this difficulty enters into most problems concerning mammals, any attempts to explain mammalian evolution by allometric growth are beset with difficulties. That many problems can be attacked in that way has been shown by Julian Huxley (*Problems of Relative Growth*, New York, 1932) who dedicated his book to Thompson.

The enthusiastic attempt by Huxley, Ray Lankaster, Gegenbaur and other fathers of modern morphology to understand the form of living things as a product of their phylogenesis has largely failed and been left unfinished. This may partially have been due to lack of material, but it is also due to insufficient analysis of the logical foundations of morphology. It would be preposterous to expect such an analysis in "Growth and Form", but it is to be regretted that Thompson does not seem to be aware of its need and by unconsciously evading the issue may have discouraged progress in this field.

In spite of its great charm (there is no other word for it), the book is not forceful. Partly, at any rate, this may be ascribed to Thompson's failure to submit his problems to a logical analysis that could compare in rigor with mathematical analysis. The book is so full of stimulating thought, however, and contains such an abundance of examples that one can feel nothing but gratitude for Sir D'Arcy Thompson for having undertaken the considerable labor of revision and re-editing. Even if the reviewer disagrees with some of its tenets, he is well aware of the fact that these differences concern mainly questions about which there is not and cannot be complete agreement. It is not a defect to have made statements open to attack, it is rather a merit to have stimulated thoughts on the fundamental problems of biology.

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